Next generation risk assessment (NGRA): bridging in vitro points-of-departure to human safety assessment using physiologically-based pharmacokinetic (PBPK) modelling - a case study with doxorubicin

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Introduction:

Chemical safety assessment is undergoing a paradigm shift from traditional animal-based toxicity testing to novel, human cell-based in vitro approaches. To bridge the gap between in vitro toxicity-pathway-based findings and in vivo human health risk, in silico approaches such as physiologically-based pharmacokinetic (PBPK) modelling are required to quantitatively translate the in vitro point of departure (PoD) or effective concentrations of test compounds into human exposure dosimetry. The physiological structure of PBPK modelling offers a scientifically-sound framework of integrating kinetic data on absorption, distribution, metabolism and excretion to predict the kinetics of the parent chemical and metabolite(s) in the target sites of the exposed organism. Comparison of key values derived from human-relevant in vitro assays, such as the highest concentrations or effect concentrations, with human plasma or tissue concentrations predicted using PBPK modelling, is key in Next Generation Risk Assessment without using animal data.

Objective:

Using the chemotherapy drug doxorubicin (DOX) as a case-study chemical for its well-known cardiotoxicity in patients, this study was aimed to investigate whether PBPK modelling can bridge the gap between information gleaned from relevant human cell assays and the risk of human health. By comparing different dose or tissue metrics to relate the exposure to toxicity, we illustrate the importance of these measures.

Method:

1. Identify in vitro DOX key concentrations or AUCs derived from two pathway-based assays as reported in literature for cardiotoxicity (Table 1), i.e. on mitochondrial homeostasis and biogenesis in A1A1 cell line (Yuan et al., 2016), and on cardia arrhythmia and cytotoxicity of DOX in hiPSC-CMs (Chaudhari et al., 2016).
2. Develop a human PBPK model (Fig. 1) of DOX and evaluate the performance of PBPK models through available clinical PK data as reported in literature (Fig. 2).
3. Collect clinical DOX cardiotoxicity-non-cardiotoxicity data in humans through literature review.
4. Predict plasma and heart concentrations of DOX under different clinical exposure settings that are related to either non-adverse or mild adverse situations using PBPK modelling.
5. Compare the plasma or heart dosimetry, as expressed in Cmax, or AUC, with in vitro assay derived key concentrations, i.e. Cmax or AUC (Fig. 3).

Table 1 Cmax and AUC values derived from the in vitro data as reported from literature

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Cell line</th>
<th>Cmax (nM)</th>
<th>AUC (nM-h)</th>
<th>Key concentration type</th>
<th>Origin of in vitro data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single: 125 nM 12-h</td>
<td>AC16</td>
<td>125</td>
<td>1500</td>
<td>PoD</td>
<td>[Yuan et al., 2016]</td>
</tr>
<tr>
<td>Single: 156 nM 48-h</td>
<td>HiPSC-CMs</td>
<td>156</td>
<td>7488</td>
<td>PoD</td>
<td>[Chaudhari et al., 2016]</td>
</tr>
<tr>
<td>Repeated: 154 nM 144-h</td>
<td>HiPSC-CMs</td>
<td>156</td>
<td>22444</td>
<td>Concentration causing functional toxicity</td>
<td>[Chaudhari et al., 2016]</td>
</tr>
</tbody>
</table>

Results:

1. The established PBPK model of DOX showed very good predictive performance (Fig. 2), which was demonstrated by the observed/simulated ratios for AUC and Cmax (within the 2-fold limits) of various clinical trials, indicating the model-predicted values are in good agreement with the respective observed values.
2. Based on the dose-response data presented in the two reported in vitro studies, key concentrations, i.e. PoD or effect concentrations expressed in Cmax and AUCs, were derived (Table 1) and used for the comparison with human exposure converted plasma or heart Cmax or AUCs.
3. Based on the systematic literature review on cardiotoxicity outcomes in cancer patients treated with DOX, we identified DOX’s dosing regimens that are related to either non (4.5mg/m2/day continuous infusion) or mild (9mg/m2/day 30 min infusion and 9mg/m2/day continuous infusion) cardiotoxicity. PBPK predictions of these three clinical settings were made on plasma or tissue AUC or Cmax, and compared with the in vitro key concentrations (Fig. 3).
4. Combined with PBPK modelling, the in vitro information obtained from toxicity pathway-based cell assays is useful in informing human cardiovascular risk of DOX (Fig. 3).
5. The heart Cmax and plasma AUC are good metrics to link in vitro findings to human risk of DOX on cardiotoxicity as in vitro PoD has shown good predictivity on human safe exposure level when these two metrics were used.
6. The DOX AUC metric appeared to be more conservative than the Cmax metric from the human safety perspective.

Conclusion:

Our study illustrates that it is possible to combine PBPK modelling of human exposure with in vitro-derived, dose-response information on toxicity to predict potential safe exposure levels in humans. The work with DOX allowed comparison with published clinical information on safe/unsafe doses in patients. The framework can pave a way towards NGRA approaches for assessing human health risk of chemicals without animal testing.

References:

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